

Photodynamic Therapy for Metastatic Melanoma Treatment: A Review

Channay Naidoo, Honors¹, Cherie Ann Kruger, PhD¹ ,
and Heidi Abrahamse, PhD¹ 

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Abstract

This review article is based on specifically targeted nanoparticles that have been used in the treatment of melanoma. According to the Skin Cancer Foundation, within 2017 an estimated 9730 people will die due to invasive melanoma. Conventional treatments for nonmalignant melanoma include surgery, chemotherapy, and radiation. For the treatment of metastatic melanoma, 3 therapeutic agents have been approved by the Food and Drug Administration: dacarbazine, recombinant interferon α -2b, and high-dose interleukin 2. Photodynamic therapy is an alternative therapy that activates a photosensitizer at a specific wavelength forming reactive oxygen species which in turn induces cell death; it is noninvasive with far less side effects when compared to conventional treatments. Nanoparticles are generally conjugated to photosynthetic drugs, since they are biocompatible, stable, and durable, as well as have a high loading capacity, which improve either passive or active photosensitizer drug delivery to targeted cells. Therefore, various photosynthetic drugs and nanoparticle drug delivery systems specifically targeted for melanoma were analyzed in this review article in relation to either their passive or their active cellular uptake mechanisms in order to deduce the efficacy of photodynamic therapy treatment for metastatic melanoma which currently remains ongoing. The overall findings from this review concluded that no current photodynamic therapy studies have been performed in relation to active nanoparticle platform photosensitizer drug carrier systems for the treatment of metastatic melanoma, and so this type of research requires further investigation into developing a more efficient active nano-photosensitizer carrier smart drug that can be conjugated to specific cell surface receptors and combinative monoclonal antibodies so that a further enhanced and more efficient form of targeted photodynamic therapy for the treatment of metastatic melanoma can be established.

Keywords

malignant melanoma, photodynamic therapy (PDT), photosensitizers, nanoparticles, passive or active targeting

Abbreviations and Acronyms

AuNP, gold nanoparticle; CTLA-4, cytotoxic T-lymphocyte antigen; Dox, doxorubicin; DTIC, dacarbazine; EPR, enhanced permeability and retention; FDA, Food and Drug Administration; mAb, monoclonal antibodies; NP, nanoparticle; PDT, photodynamic therapy; PS, photosensitizer; ROS, reactive oxygen species; UV, ultraviolet; 5-ALA, 5-aminolevulinic acid.

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Introduction

Cancer

Cancer is caused by environmentally induced gene mutations, which in turn trigger cells to proliferate at an abnormally rapid pace.¹ These rapid abnormal proliferations of the cells produce either benign or malignant tumors.² Cancer classification is determined by 4 factors: the type of cell which the tumor resembles, the tumors origin, the stage of the tumor, and the current location of the tumor.³

Malignant tumors often spread to surrounding tissues and move throughout the body using circulatory or lymphatic

systems, causing metastasis.⁴ Due to the ability of cancer to metastasize, this makes localized treatment redundant and therefore problematic in the annihilation of the cancer cells.⁵

¹ Laser Research Centre, Faculty of Health Sciences, University of Johannesburg, Johannesburg, South Africa

Corresponding Author:

Heidi Abrahamse, PhD, Laser Research Centre, Faculty of Health Sciences, University of Johannesburg, P.O. Box: 17011, Doornfontein 2028, South Africa.
Email: habrahamse@uj.ac.za



Due to the amount of new cases diagnosed annually, cancer is one of the most predominant health threats to individuals.⁶ There are multiple conventional cancer treatments available such as surgery, chemotherapy, and radiotherapy or a combination; they are reliant on the type, location, and stage of the cancer.⁷ Additionally, these treatments often are invasive and induce severe side effects in patients.³ Thus, the investigation into alternative forms of treatment need to be executed in order to develop new therapies that can possibly mitigate these unwanted side effects.³

Metastatic Melanoma

Skin cancers are identified and named according to the cell from which they originated from as well as their clinical behavior.⁸ The 3 general types of skin cancer are basal cell carcinomas, squamous cell carcinomas, and cutaneous malignant melanomas.⁹ The first 2 types are commonly referred to as nonmelanocytic or noninvasive skin cancer, since they don't originate in skin melanocytes and don't spread to surrounding healthy tissues.¹⁰ However, cutaneous malignant melanomas tend to spread to surrounding tissues and so are considered to be metastatically invasive.¹¹

Melanoma is an invasive and aggressive form of skin cancer; which is known for its elevated multidrug resistance, very low rate of patient survival, and tendency to relapse with ease.¹² According to the Skin Cancer Foundation, it is estimated that in 2017 roughly 87 110 new cases of metastatic melanoma will be diagnosed within the United States alone and that an estimated 9730 people will die from it due to its invasiveness.¹³

Melanoma originates in the deepest regions of the epidermis and in the beginning regions of the dermis, where melanocytes that produce melanin pigment are located.⁵ Thus, it develops from a single melanocyte that is either malignantly transformed or by the dysfunction of dysplastic nevi.¹⁴

Metastatic melanoma is considered to be a late form of stage IV of skin cancer and occurs when cancerous cells in the epidermis metastasize and progress to other organs of the body that are located far from the original site.⁵ It is crucial to diagnose melanoma in its early stages before it metastasizes, as once it has spread, it is difficult to locate its origin and so treatment and patient's survival rate tends to be hindered.¹⁵

The most common cause of melanoma is attributed to ultraviolet radiation (UV) exposure, family history, and personal history of melanoma.¹⁶ In 2016, the World Health Organization reported that the incidence of skin cancer is on the rise due to the excessive UV rays that individuals are being exposed to. Additionally, lighter skinned patients who have lack of skin pigmentation have a much higher risk of getting nonmelanoma or melanoma skin cancers than compared to dark-skinned patients, due to their increased risk of UV-induced sunburn skin damage.¹¹

Conventional Treatments for Metastatic Melanoma

Prognoses of metastatic melanoma are performed by utilizing a staging classification system that assesses and describes the degree of disease development in patients (AJCC, American Joint Committee on Cancer).¹⁷ The main factors of this staging system are location of the primary tumor; tumor size, number of tumors, lymph node involvement; and the absence or presence of metastasis.¹⁸ In order to determine the stage of cancer, assessments such as physical examinations, imaging tests, laboratory tests, and pathology reports are performed on patients.¹⁷ Conventional treatments for metastatic melanoma include surgery, chemotherapy, radiation, and biological therapy.⁵

Surgery

The primary treatment for melanoma is surgery, whereby the lesion is excised with some of the unaffected surrounding tissues to ensure all the affected tissue is removed and no cancerous cells are present in the area to proliferate.⁵ Surgery offers the best chance of recovery if the melanoma has been diagnosed within its early stages and has not yet had a chance to metastasize.¹⁹

Chemotherapy

The next conventional treatment for cancer is chemotherapy, which has the ability to alleviate, control, or completely cure skin cancer; its success is dependent on the patient's severity of the cancer at time of diagnosis.²⁰

Chemotherapy relies on effective drugs to stop cancer cells from proliferating abnormally or to slow down their overall growth rate.²¹ Metastatic melanoma chemotherapeutic drugs include dacarbazine (DTIC), paclitaxel, platinum compounds, and temozolomide.²² According to Tang *et al*,²³ malignant melanomas show <20% response rate to these types of drugs due to various resistance mechanisms. Chemotherapeutic drugs may be administered orally, via injection, intraperitoneal, intra-arterial, topically, or intravenously.²³ The drawback to chemotherapy is that it also causes damage to healthy cells as well as severe side effects in patients such as fatigue, secondary infections, anemia, nausea, vomiting, and constipation.²⁴ Thus, chemotherapy sessions are generally spread out during a period of time to allow patients' bodies to recover between treatments.²⁵ Chemotherapy can solely be administered to patients; it is usually administered either after surgery or in combination with other treatments such as radiation or biological therapy.²⁰

Radiotherapy

Radiotherapy is another therapy that is used for the treatment of melanoma.²⁶ It is similar to chemotherapy in the sense that it can alleviate, control, or cure cancer depending on the severity and type of cancer the patient has been diagnosed with.²⁷ In this type of therapy, radiation is employed to annihilate cancer cells

through external or internal administration.²⁸ With internal administration, radiation is precisely administered only to the affected area of a patient's body, whereas with external radiation the beam is applied to a much wider area and so is considered less precise.²⁸ Radiation therapy causes side effects such as skin changes, fatigue, and nausea as well as affects healthy surrounding tissues.²⁴ Depending on the severity and type of cancer, a patient can undergo radiation therapy that may be applied in combination with chemotherapy, and this often induces far harsher side effects.²⁹

Biological Therapy

Biological therapies also rely on drugs to cure cancer.³ Biological therapies differ from chemotherapy, since the drugs that are administered to patients aid the immune system in combating the cancer rather than just directly killing rapidly proliferating cells.³⁰ This type of therapy is often used in combination with other therapies.³ Currently, the Food and Drug Administration (FDA) has only approved 3 conventional biological agents for the treatment of advanced metastatic melanoma: DTIC—approved in 1975; recombinant interferon α -2b—approved in 1995; and high-dose interleukin 2—approved in 1998.³¹

Unconventional Treatments for Metastatic Melanoma

Molecular-Targeted Therapy and Immunotherapy

Molecular-targeted therapy uses anti-cytotoxic T-lymphocyte antigens (CTLA-4) antibodies to target CTLA-4; they are over-expressed on activated T-lymphocytes and so act as a negative regulator of T-cell activation.³² This enhances the immune system's ability to destroy cancer cells.³² This type of immunotherapy treatment targets programmed death 1 and programmed death ligand 1 or 2 as well as CTLA-4 in metastatic melanoma cancer cells.³³ The problem associated with this type of therapy is that the overall treatment is not effective for all patients, as it is influenced by immune-related side effects and resistance factors.³⁴ Studies performed by Cirenajwis *et al*³⁵ evaluated the effects of ipilimumab as an anti-CTLA-4 inhibitor to effectively treat metastatic melanoma in patients; however, severe side effects were noted. Thus, in order to make this type of malignant melanoma treatment more efficient, it is essential to improve the molecular targeting abilities of the treatment, as well as overcome resistance.³⁶

Nanodrugs

Recent advances in research have exploited the use of nanotechnology for the treatment of cancer; this enhances targeted cancer cell drug delivery and uptake and drastically reduces their overall cytotoxic side effects to normal tissues.³⁷ Some nanodrugs have already been FDA approved for use in preclinical and clinical trials, as they have been shown to either target

and directly kill tumor cells or improve overall targeted chemotherapy drug delivery.³⁸

Photodynamic Therapy

Photodynamic therapy (PDT) has been investigated for the past 30 years as an unconventional treatment for cancer.³⁹ It involves the administration of photosensitizer (PS) light-sensitive drug to targeted cancer cells, and the localization of laser light at an appropriate wavelength is used to excite the PS.⁴⁰ The excitation of the PS causes the production of cytotoxic reactive oxygen species (ROS), such as singlet molecular oxygen, hydroxyl radicals, and/or superoxide anions, which achieve photocytotoxicity through oxidatively stressing cancer cells and so induces damage to their cellular biomolecules (ie, lipids, proteins, and nucleic acids), rendering them inactive.⁴¹

This unconventional form of treatment is less invasive than conventional forms of cancer treatment; it specifically targets a cancerous tumor region and so produces localized destruction with limited side effects.⁴²

Mechanism of PDT action. There are 2 types of action mechanisms in PDT, which occurs in an oxygen-dependent environment. Both types produce oxygen; however, type 1 reactions produce superoxide anion radical, whereas type 2 produces a singlet oxygen.⁴³ Factors that determine this mode of action includes: PS concentration, PS localization, amount of adenosine triphosphate within the cell, the genetic makeup of the cell as well as the fluence and wavelength of laser light exposure.⁴¹

The modality of PDT, as shown in Figure 1, entails a PS that is activated at a specific wavelength inducing excitation. In the excited state also known as a triplet state, 2 types of reactions occur. In type I reaction, a superoxide anion radical is produced, and these interact with oxygen to produce oxygenated products. In type II reactions, the triplet can transfer its energy directly to the oxygen, therefore producing a singlet oxygen; it is considered a highly ROS.⁴³

Mechanism of PDT facilitated cell death cytotoxicity. During the mechanism of PDT action, the ROS that is generated induces an apoptotic, autophagy, and/or necrotic mode of cell death (Figure 2).⁴⁴ Factors that influence the mode and degree of cell death include cellular morphology, immunological responses, enzymatic activity, light wavelength and intensity, oxygen concentration, and PS physiochemical characteristics as well as PS subcellular location.⁴⁵ These factors determine whether the mode of cell death is nonprogrammed or programmed.⁴¹

Apoptosis is a programmed mode of cell death that is usually characterized by membrane and nuclear degradation.⁴⁶ The PSs generally tend to localize in cellular mitochondria when this form of cell death occurs, and it is the most common associated mode of cell death in PDT.⁴⁷ Apoptosis in target cells is activated by specific signals that trigger a variety of pathways to commit suicide in response to these signals.⁴⁸ As the pathways collapse, protein caspases are activated to degrade cellular contents such as nucleic and polypeptide

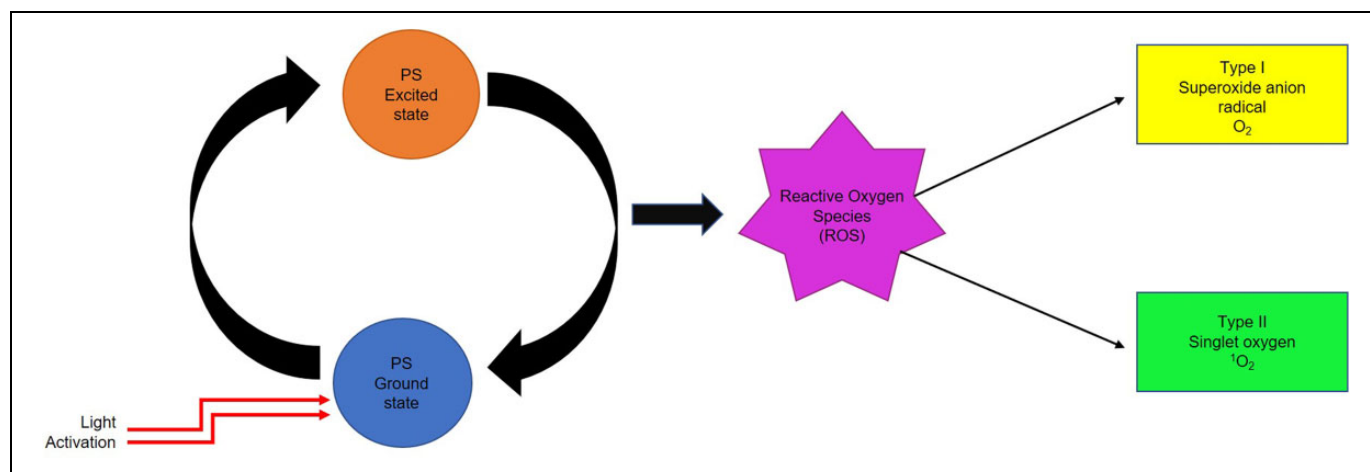


Figure 1. The electron and energy transfer during photodynamic therapy (PDT).

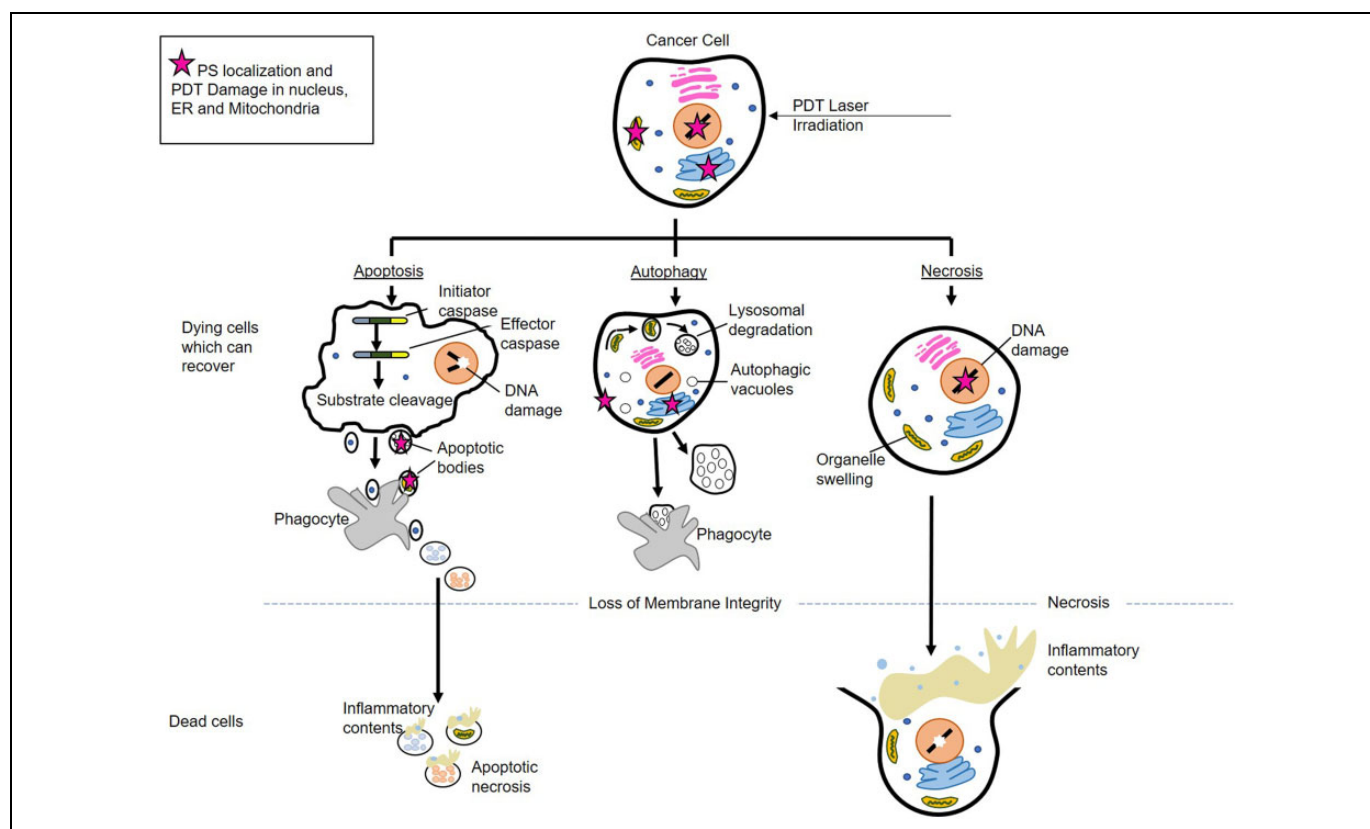


Figure 2. Different forms of cell death that can be induced in photodynamic therapy (PDT) cancer treatments, namely, apoptosis, autophagy, and/or necrosis.

material.⁴⁹ Therefore, apoptosis is a regulated process that is induced.⁵⁰

Necrosis is a nonprogrammed mode of cell death that is characterized by inflammatory responses, which are initiated from external stimuli such as infections or trauma.⁵¹ The PS that induces necrosis tend to localize within the plasma membrane of target cells.⁵² Necrotic cell death pathways events involve membrane permeability, movement of calcium ions

across the endoplasmic reticulum, cytoplasmic swelling (oncosis), calcium-dependent calpain activation, lysosomal rupture, followed by the breaking down of cell component, and overall induction of inflammatory responses.⁵³ Within eukaryotic cells cell death, it is regulated by transduction and catabolic activities that use receptor interacting proteins.⁵⁴ Photodynamic therapy-induced apoptotic modes of cell death can sometimes be converted to necrosis when conditions such as a high

concentration of PS is administered to target cells or very high fluencies are used to excite the PS.⁵⁵ These can cause the cell to rapidly disintegrate and die when compared to apoptotic programmed cell death.⁵⁵

However, recent studies by Dewaele and colleagues⁵⁶ have noted that after PDT irradiation of certain PSs, another mode of cell death known as autophagy can be induced. Photodynamic therapy-induced autophagy occurs when a cell attempts to repair itself to overcome photoinjury; however, if this response fails then the cell is signaled for programmed apoptosis.⁵⁷

Photodynamic therapy challenges. Some challenges faced when PDT is applied to cancer treatments (to ensure its effectiveness) include applying the correct wavelength and exposure time to maximally excite a specific PS to ensure the highest yield production of ROS.⁵⁸ Additionally, the concentration and localization of PSs, which is taken up by target cells, is important to ensure that maximum levels of ROS can be generated to induce maximum cell death.⁵⁹ If passive diffusion via the enhanced permeability and retention (EPR) effect is utilized as a mode of PS drug uptake, PSs do tend to localize more predominantly in rapidly proliferating tumor cells; however, they also tend to be absorbed by some healthy surrounding tissues that cause unwanted side effects.⁶⁰ Thus, to improve PS tumor selectivity, as well as the overall efficiency of PDT research, research nowadays tend to focus more on the development of multi-component PS drug targeting strategies that enhance PS delivery and concentration in only specific targeted cells.⁶¹ Finally, sometimes within PDT applications, the ability to access deep-seated tumors with laser light is problematic, and so alternative measures and treatments need to be considered for application in combination with PDT.⁶²

Photodynamic therapy has been successfully used for the treatment of basal cell carcinoma head and neck cancers which are over exposed and so easily accessed by laser light irradiation.⁶³ However, skin cancers that have internally metastasized are far harder to treat with PDT, since they receive far less exposure to laser light irradiation.⁶⁴ Additionally, metastatic melanomas are pigmented with melanin, which does not allow for efficient laser light to reach the target sight; hence, PDT treatment for this form of skin cancer is often less effective.⁶⁵ Nevertheless, recent research developments are currently focused on developing targeted cellular uptake photosynthetic drugs, which can be activated by a far higher wavelength with deeper tissue penetration and improved ROS generation as well as far more compacted lasers that can deliver light endodermally to overcome these issues.⁶⁶

Effective PSs Used for Metastatic Melanoma PDT Treatment

There are different classes of PSs that have been investigated over the years for PDT treatment of metastatic melanomas.⁶⁷ When considering which type of PS to apply to a particular PDT treatment, there are a number of factors that need to be

considered such as its characteristics, its mode of action, where it localizes as well as what type of cell death it induces.⁶⁸

Generally, most PSs tend to localize in most cellular organelles other than the nucleus and so are less likely to induce carcinogenesis, DNA damage, or mutations.⁶⁹ The PSs that are used for PDT applications are divided into 3 generations, which is dependent on their photochemical and photophysical characteristics in relation to their cellular mode of action.⁷⁰ First-generation PSs tend to induce vascular tissue damage as localization, with severe side effects, indicating that their specific localization in target cells is limited.⁷¹ Second-generation PSs tend to cause only tumor cell cytotoxicity, suggesting a more passive form of PS localization in organelles such as mitochondria, lysosomes, endoplasmic reticulum, and plasma membrane.⁷² Therefore, the side effects induced by second-generation PSs are far less than those of first-generation PSs.⁷² Third-generation PSs are photosynthetic drugs that have been further functionalized by the addition of various targeting biomolecules to enhance their specific cellular drug uptake and absorption.⁷³

The 4 main classes of PSs include porphyrins, phthalocyanines, chlorins, and porphycenes.⁷² Porphyrins have been used excessively in PDT applications, as they are very stable, however, are of first generations, and so tend to induce photosensitivity and tissue penetration depth is poor.⁷⁴ Chlorins are second-generation PSs that are reduced from porphyrin or chlorophyll derivatives.⁷⁵ Reports by Jerjes and colleagues⁷⁶ have noted that chlorins have a high PDT efficacy rate when treating basal cell carcinomas and squamous cell carcinomas. Phthalocyanines are second-generation PSs, which have an even higher PDT efficacy, as they contain a diamagnetic metal ion that allows for deep laser light tissue penetration with far less phototoxic side effects.⁷⁷ Porphycenes are electronic isomers of porphines that are synthetically produced and so require further investigation into their mode of action as at present it is not fully understood.⁷⁸ Table 1 reports on current PSs that have been investigated and applied for the PDT treatment of metastatic melanoma as well as lists the functional parameters and outcomes of each study. After the review of Table 1, it was concluded that the most common PSs that have been investigated for the PDT treatment of metastatic melanoma include those from the phthalocyanines and porphyrin PS classes; however, in general, metallophthalocyanine PSs seem to be more promising for the treatment of metastatic melanoma than porphyrins, as they noted overall less photosensitivity/phototoxicity.

Nanotechnology and Nanoparticles

Nanotechnology in research has been shown to have an extremely promising future in cancer drug delivery mechanisms.⁸⁷ This is due to the fact that nanostructures have a large surface area to volume ratio, allowing drugs to be bound to nanoparticles (NPs), which act as carriers that promote cellular uptake.⁸⁸ Additionally, properties of NPs can be engineered to exhibit certain properties to assist in drug delivery such as: the

Table 1. The Outcomes and Parameters of PSs' Used During PDT to Treat Melanoma.

Photosensitizer (PS)	Parameters	Cells	Result	Reference
Verteporfin	Wavelength: 690 nm; Fluency: 520 mJ/cm ² ; (PS): 5.5 µmol/kg	Melanoma tumors in mice	Large necrotic areas were seen in tumor and reduction in tumor growth was observed. The photosensitivity of Verteporfin is dose-dependent as higher doses yield prolonged photosensitivity.	5
10,15,20-tritolyldiporphyrin-5-(4-amidophenyl)-[5-(4-phenyl)-10,15,20-tritolyldiporphyrin] (T-D)	Wavelength: 630 nm; Fluency: 81 J/cm ² ; (PS): 10 ⁻⁷ M	Human melanoma cells (SK-MEL 188); Mouse melanoma cells (S91)	Both types of cells showed a 3-fold decrease in size compared to control cells. It requires high-energy irradiation for phototoxicity but has more advantages than Photofrin.	79
5,10,15,20-tetrakis(2,6-difluoro-3-N-methylsulfamoylphenyl) bacteriochlorin	Wavelength: 633 nm; Fluency: 6.2 J/cm ² ; (PS): 20 µM	Mouse melanoma cells (S91)	S91 cells still destroyed 24 hours post treatment using vascular-targeted PDT. Cellular-targeted PDT led to strong pO ₂ compensatory effects and tumor regrowth.	80
Halogenated porphyrins	Wavelength: 633 nm; Fluency: 10 J/cm ² ; (PS): 10 µM	Human melanoma cells (A375)	PS showed a 30-fold increase in killing efficiency than when compared to Photofrin, since its halogenated structure interfered with P-glycoproteins. All porphyrins present a much higher phototoxicity than Photofrin.	81
Meso-tetrakis-(4-sulfonatophenyl) porphyrin (TPPS ₄)	Wavelength: 633 nm; Fluency: 10 J/cm ² ; (PS): 12.5 mg/mL	Human melanoma cells (G361)	Most effective sensitizer is ZincTPPS ₄ , since the IC ₅₀ value was 12.5 mg/mL at the dose of light radiation of 10 J/cm ² . According to the results, ZincTPPS ₄ seems to be more phototoxic than TPPS ₄ .	82
5-aminolevulinic acid (5-ALA)	Wavelength: 420-1400 nm; Fluency: 45 and 90 J/cm ² ; (PS): 200 g/mL	Mouse melanoma cells (Mel25)	Effectively killed cells <i>in vitro</i> , however reported minor effects when tested <i>in vivo</i> .	83
Ruthenium porphyrins	Wavelength: 652 nm; Fluency: 5-30 J/cm ² ; (PS): 10 µM	Human melanoma cells (Me300)	A significant cell death ranging from 60% to 80% decrease in cell viability was noted. It shows some degree of cytotoxicity in the dark but seems to present no phototoxicity upon irradiation.	84
Phthalocyanine	Wavelength: 630-780 nm; Fluency: 10 J/cm ² ; (PS): 2 × 10 ⁻⁹ M	Achromic melanoma cells (M6)	Significant photo-killing was observed in cultured cells that was linked to lipid peroxidation.	85
Metallophthalocyanine (MPc) and 5-aminolevulinic acid (5-ALA)	Wavelength: 680 nm; Fluency: 10 J/cm ² ; (PS): 4 mM of 5-ALA and 10 mM MPc	Human metastatic cells (A375)	Significant decreases in cell viability ranging from 60% to 80% was reported, with a cytotoxic induction of apoptotic cell death. However, control cells which received 5-ALA only noted photo toxicity before irradiation, whereas cells that received MPc did not.	86

Abbreviations: MPc, metallophthalocyanine; PDT, photodynamic therapy.

diameter control, stability, permeability, porosity, and hydrophilic adaptations.⁸⁹

Applications of NPs within cancer PDT therapy PS drug delivery systems are fast becoming effective; they are easy to synthesize, have high surface area-to-volume ratio (they have the ability to support a large amount of therapeutic agents), and have simple surface chemistry with the possibility of functionalization.⁹⁰ Additionally, due to the small dimensions of NPs, they can easily accumulate in cells, more specifically in tumor cells due to the EPR effect.⁹¹ The EPR allows NP drug carriers to enter tiny spaces between tumor cells, suppressing lymphatic filtration and so the drug uptake in tumor cells is increased.⁹²

The factors that can affect the EPR are the pore dimensions for the molecule to enter at the tumor site, the tumor location, the size of the tumor, and the type of tumor which is present; optimizing NPs as carriers for drug delivery is essential.⁹³

Thus, the incorporation of antibodies or targeting molecules to NPs can promote PS drug attachment to malignant cell membranes, cytoplasmic receptor sites, and nuclear receptor sites, which increases drug uptake in specific tumor cells while reducing the overall toxicity in healthy cells.⁹⁴ Additionally, engineered NPs allow compatibility with the immune system and therefore tend to go by unnoticed by immune system barriers, as they mimic biological molecules and can combine to

other molecules such as PSs that improve and enhance drug delivery.⁹⁰ Moreover, NPs can be further functionalized into active targeting molecules through the attachment of molecules that are specifically compatible to targeted tumor cells.⁹⁵

However, when it comes to pharmaceutical nanotechnology cancer drug engineering delivery, researchers need to take the following into consideration such as safety, bioethical issues, toxicity hazards, and physiological issues. Thus, scientific researchers must take the following into consideration when designing functionalized nanotechnology-based drug delivery systems such as size, characterization, and specific targeting of diseased tissue only, by selecting antibodies or other means of selective binding which are only overexpressed in definitive tumor cells so as to enhance drug delivery and reduce overall nonspecific toxicity.⁹⁶

Nano-Drug Delivery Carrier Platforms and Targeting Strategies for PDT Cancer Treatment

For effective PDT, functionalized NP platforms need to be used in order to enhance PS drug delivery, and each type has its own individual advantages, whether it may be passively or actively absorbed by tumor cells (Table 2; Figure 3).⁹⁷ These strategies enable PSs that are delivered to tumor sites to induce cell death.⁹⁸ This type of drug delivery needs to be targeted to ensure that the PS is only delivered to the tumor target site and not healthy surrounding tissues to prevent phototoxicity and unwanted side effects in healthy cells.⁹⁹

Passive PS absorption is accomplished when the drug accumulates in tumor cells due to NP characteristics such as composition and size, and overall drug uptake is only affected by the surrounding tumor environment (such as hypoxia or low pH) and EPR effect.¹⁰⁰ Examples of NP drug delivery platforms, which passively enhance PS drug accumulation in PDT applications include: micelles and liposomes, polymeric particles, dendrimers, metal oxide, ceramic, silica, and alumina organic-based NP.¹⁰¹

In active absorption, the PS drug is delivered to a specific target tumor site through a molecular recognition process.¹⁰² The NPs are functionalized with target molecules that specifically bind to receptors overexpressed by tumor cells, leading to enhanced PS drug uptake.¹⁰³ Targeting molecules that are exploited in targeting PS drug delivery to tumor cells include: monoclonal antibodies (mAb), aptamers, antibody fragments, peptides, and/or DNA/RNA.¹⁰⁴ Examples of NP drug delivery platforms that actively enhance PS drug targeting in PDT are generally inorganic nanomaterials such as: quantum dots, solid lipids, self-illuminating nanocrystals, theranostic, hydrogels, immune-conjugates, metal-oxide based or upconverted.¹⁰⁵

However, studies by Maeda⁹³ have shown that PDT PS carrying NPs that use a passive targeting strategy tend to sometimes affect healthy surrounding tissues more than active targeting strategies, since passively absorbed NP drugs cannot

Table 2. NP Platform Passive or Active Drug Carrier Systems, With Strategic Advantages for PDT Cancer Treatment.

NP Platform	Advantages
Passive PDT PS Tumor Drug Absorption	
Micelles and Liposomes	Enhanced tumor uptake (Liposomes) and improved tumor phototoxicity (micelles) ⁸⁷
Polymeric particles (polyethylene glycol)	High drug loading, biocompatibility, high drug encapsulation, and better drug release profile ^{108,109}
Dendrimer encapsulated NP	High drug loading ¹¹⁰
Metal oxide NP	Higher loading capability, biocompatibility, and surfaces can be easily modified with different functional groups and nontoxicity ¹¹¹
Ceramic	Highly stable, biocompatible, and hydrophilic ¹¹²
Silica	Surfaces can be easily modified with different functional groups ¹¹³
Alumina	Highly stable and induce oxidative stress ¹¹⁴
Active PDT PS Tumor Drug Absorption	
Quantum dots	Large absorbance cross section and size-tunable optical properties ¹¹⁵
Solid lipid	Improved stability, better drug release, high loading capability, and biocompatible ^{116,117}
Self-illuminating nanocrystals	Uses lower doses of radiation ¹¹⁸
Theranostic (biodegradable photoluminescent poly)	Strong fluorescence and cytocompatibility. Can be conjugated with peptide to increase loading efficiency ¹¹⁹
Hydrogels	High absorption capability and highly stable and durable ¹²⁰
Immuno-NP	Highly specific molecule, improves drug release within desired cell ¹²¹
Cerium oxide, zinc oxide, copper oxide	Highly selective, radioprotective, size-tunable optical properties, and nontoxic ¹²²⁻¹²⁶
Upconverting	Near-infrared optical absorption coefficients ¹²⁵

Abbreviations: NP, nanoparticle; PDT, photodynamic therapy; PS, photosensitizer.

exclusively differentiate between cancerous and normal cells and so occasionally distribute in healthy tissues. Thus, to improve tumor PS drug accumulation specificity and limit unwanted side effects, recent research has now focused on synthesizing specifically targeted activity absorbed NP–PS bioconjugates for PDT cancer applications.¹⁰⁶ However, to date, this still remains a challenging task as the overall NP–PS drug delivery is dependent on the size, surface functionalities, and specificity of NP carrier, as well as the NP disintegration and PSs drug release rate once absorbed by specifically targeted cells.¹⁰⁷

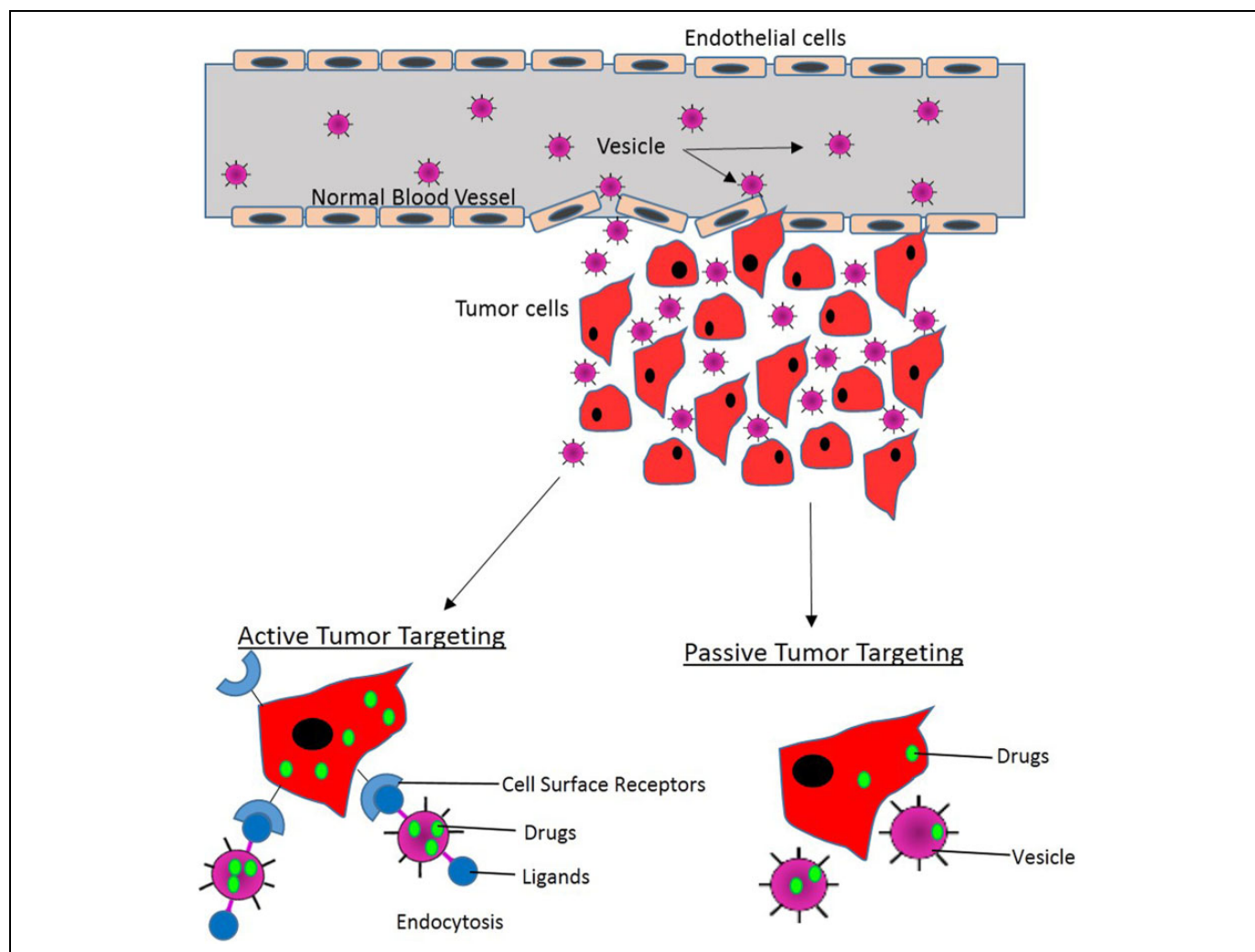


Figure 3. Passive and active forms of photosensitizer (PS) nano-drug cancer targeting strategies used in the photodynamic therapy (PDT) treatment of cancer.

Nanotechnology and Metastatic Melanoma

In terms of the utilization of nanotechnology for the PDT treatment of skin cancer, topical drug delivery can be improved through NP engineering by understanding the NP drug mode of delivery and skin interaction.¹²⁷ The delivery of topical drugs is achieved through 3 different skin sites that include open hair follicles, furrows, and the stratum corneum surface.^{102,128} The skin can become damaged through various factors such as aging and disease; this in itself is a potential and ideal route for drug delivery.¹⁰² In studies performed by Naves and colleagues,^{129,130} it was found that microemulsions that contained 5-fluorouracil, applied topically allowed an enhanced drug absorption in patients diagnosed with squamous cell carcinoma that had ulcerations on their skin surface. Studies by the Scientific Committee on Consumer Products reported that NPs which are larger than 20 nm in diameter, cannot reach viable tissues, however can deeply penetrate hair follicles, whereas NPs that are less than 10 nm in diameter can penetrate the skin and reach viable tissue.¹²⁸ Although NPs

tend to interact in an adherent way with the skin, careful consideration needs to be taken when engineering NP drug delivery systems in terms of NP size, tumor location, and mode of delivery to ensure maximum PS drug accumulation occurs only at the target site.¹¹³

Gold NPs (AuNPs) have been extensively investigated in PDT-induced cancer treatments, as they have tunable optics and photothermal properties, which allow for the conversion of laser light into heat improving targeted cellular destruction.¹⁰⁵ Studies Baldea and Filip¹³⁰ noted that 5-ALA PS drugs were effectively absorbed and taken up in a 3-fold higher concentration within *in vitro* cultured murine melanoma tumors than when compared to the photosynthetic drug administration alone, suggesting that the AuNPs showed overall enhancement of cellular PS drug uptake. Studies by Brys *et al*¹³¹ investigated the clinical outcomes in patients having melanoma by administering pegylated liposomal nanocarriers that were conjugated to Doxorubicin (Doxil®, USA). The study revealed that the uptake of Doxil, which is an FDA-approved anticancer drug,

Table 3. Passive NP Platform PS Drug Carrier Systems, With Resulting Outcomes for PDT Treatment of Metastatic Melanoma.

Conjugated PS	Passive NP Platform	Result	Reference
Phthalocyanine	Silica NP	Particle size was 28 nm, absorption at 674 nm, reduced and delayed photobleaching and high efficiency in generation of reactive oxygen species.	132
Verteporfin	Silica NP	UV-Vis spectrum showed bands at 425 nm, red light induce singlet oxygen release and Ver-Mesoporous silica nanoparticles irradiation resulted in cell line SK-MEL 28 proliferation halving whereas the same treatment when NP's internalization was inhibited resulted in 30% reduction in cell growth.	133
5-aminolevulinic acid (5-ALA)	AuNP	A .023 <i>P</i> value was obtained between 5-ALA and the PS-NP conjugate in fluorescence intensity, cell survival % at (2 mM) of AuNPs showed a significant difference to (0.25 mM) and (0.5 mM) AuNP. Maximum cell death was obtained with 60 J/cm ² of irradiation.	134
None	AuNP loaded with rose bengal (RB) and doxorubicin (Dox)	AuNP were found to enhance the singlet oxygen generation rate, with a maximum enhancement factor of 1.75. Gold-loaded liposomes containing RB and Dox where Dox release was triggered by light were found to exhibit higher cytotoxicity compared with the liposomes loaded with RB and Dox alone.	135
None	Mesoporous-silica NP loaded with dacarbazine	<i>In vitro</i> , nanocarrier exhibited the strongest cytotoxicity to melanoma cells compared with DTIC-NP and free DTIC. No <i>in vivo</i> studies performed yet.	136
Hydrophilic chlorine	Magnetic NP	Cell viability measurements demonstrated that PS-MNPs were more phototoxic than PEI-chlorin <i>p6</i> against 2 variants of B16 murine melanoma	137
None	Albumin-stabilized paclitaxel	Improved progression-free survival compared with dacarbazine treatment alone	138
None	Albumin-stabilized paclitaxel NP mixture loaded with carboplatin	Improved overall survival compared to ipilimumab	138
5-aminolevulinic acid (5-ALA)	Chitosan NP	Improved stability, enhanced delivery, and superior PDT phototoxicity	139

Abbreviations: AuNP, Gold NPs; DTIC, dacarbazine; NP, nanoparticle; PDT, photodynamic therapy; PS, photosensitizer; UV, ultraviolet radiation.

was improved with enhanced toxicity than when compared to the standard Doxil uptake control studies that had no nanocarrier assistance.¹³¹

Nano-PS Drug Targeting Strategies for PDT Metastatic Melanoma Treatment

Table 3 lists the various types of passive nano-PS drug delivery carrier platforms that are currently under investigation for the PDT treatment of metastatic melanoma as well as the resulting outcomes of these studies. After review of the result findings of Table 3, it can be concluded that in general metastatic melanoma PDT studies have tended to focus on the conjugation of porphyrins, phthalocyanines, chlorin PSs to gold, magnetic, silica, and albumin-stabilized passive NP platforms.

In terms of active nanodrug delivery systems to improve the specific uptake and targeted delivery drugs to metastatic melanoma tumor sites, various NP drug delivery platforms are currently under investigation, which are functionalized with mAbs, antibody constructs, or small molecule inhibitors (Table 4).¹⁰² These active NP drug delivery systems are specifically directed at metastatic melanoma cell surface receptors or target components of the intrinsic signaling pathways of cells to enhance various forms of treatment.

Studies performed have noted that metastatic melanoma cells tend to overexpress integrin $\alpha v\beta 3$, extracellular matrix 1, a combination of *Drosophila* protein and *Caenorhabditis elegans* protein, B-cell lymphoma 2, mitochondrial p32 protein, integrin alpha 4 beta 1 protein, ephrin type-A receptor 2, and TRAIL-receptor 2 on their cell surface receptors.²³ The protein melanoma inhibitory activity was identified as a key component that was involved in the progression and metastasis of malignant melanoma.¹⁴³ Thus, active NP-PS smart drugs can possibly be synthesized with mAbs, which bind using a lock-and-key mechanism to these specifically overexpressed cell surface antigen receptors, ensuring PS drugs are delivered to tumor target sites only and not healthy surrounding tissues.¹⁴⁴ Currently, rituximab, bevacizumab, and trastuzumab are mAbs that are FDA approved and utilized to target metastatic melanoma cells.¹⁴⁴ However, mAb nanotargeting smart drugs are very expensive to use, and large-scale production is problematic and challenging due to their physical and chemical properties that have to undergo detailed characterization and additionally to ensure that during manufacturing the process, composition and structure is not altered as this could have adverse effects.¹⁴⁴

It can be observed from Table 4 that no current PDT studies have been performed in relation to active NP platform PS drug

Table 4. Active NP Platform PS Drug Carrier Systems, With Resulting Outcomes for PDT Treatment of Metastatic Melanoma.

Conjugated PS	Active NP Platform	Result	Reference
None	Albumin-stabilized paclitaxel NP mixture loaded with VEGF inhibitors and carboplatin	Improved overall survival compared with patients treated with VEGF inhibitors and temozolomide	138
None	Anti-RRM2 siRNA-loaded cyclodextrin polymer-based NP's, targeted to transferrin-overexpressing cells	Successful reduction in RRM2 expression in tumor tissue from treated patients	138
None	Liposomes used to target melanoma cells that express integrin $\alpha v \beta 3$ loaded with tetraiodothyroacetic acid	Increased cellular uptake by 98.5%, with apoptotic cell death.	23
None	NP that target cell penetrating peptide RGD loaded with curcumin	Study showed that active targeting NP inhibited tumor growth significantly when compared to passive NP drug delivery	132
None	Albumin-stabilized paclitaxel NP mixture loaded with bevacizumab and ipilimumab mAb	Clinical trials in patients with metastatic melanoma was used as a first approach therapy with poor efficacy, for patients who could not have their tumors surgically removed.	140
None	DTIC-NPs-DR5 mAb loaded with DTIC and TRAIL-receptor 2 (DR5) mAb	Actively targets and induces apoptosis. DTIC-NPs-DR5 mAb showed significantly enhanced cytotoxicity and increased cell apoptosis in DR5-positive malignant melanoma cells.	141
None	Silver nanoparticles mixture loaded with GKRR peptide	Enhances uptake ratiometric measurements, we were able to classify the PPC-1 cell line as mainly NRP-1-positive, with $75\% \pm 5\%$ R-AgNP uptake, and the M21 cell line as only p32-positive, with $89\% \pm 9\%$ K-AgNP uptake.	142

Abbreviations: AbNp, silver nanoparticle; DTIC, dacarbazine; mAb, monoclonal antibody; NP, nanoparticle; PDT, photodynamic therapy; PS, photosensitizer; RGD, arginylglycylaspartic acid; VEGF, vascular endothelial growth factor.

carrier systems for the treatment of metastatic melanoma. Thus, this type of research requires further investigation into developing a more efficient active nano PS carrier smart drug that can be conjugated to targeting molecules and combinative mAbs so that a further enhanced and more efficient form of targeted PDT for the treatment of metastatic melanoma can be established.

Conclusion

In recent years, the incidence and mortality rates of metastatic melanoma are on the rise due to patients being excessively exposed UV sun rays, as the atmosphere slowly disintegrates. Currently, metastatic melanoma remains a very difficult form of cancer to cure, and overall the findings from this review suggest that neither conventional nor unconventional treatments used in singular approaches are promising.

Recent research is showing promising results in terms of using combination therapeutic treatments with actively specific NP platform carrier systems which can target metastatic melanoma tumors. This type of research needs further investigation in terms of PDT applications, whereby PS that have previously been examined (Table 1) for metastatic melanoma, are conjugated to various NP platforms (Tables 2 and 4), which have been functionalized with mAbs, antibody constructs, or small molecule inhibitors to effectively enhance the active uptake of photosynthetic drugs in metastatic melanoma cells, increasing its concentration and overall induced PDT cell death within tumor cells only, with limited side effects.

Presently, there are some newly developed conventional therapeutic agents in preclinical trials; however, the search for

the cure of metastatic melanoma remains ongoing and actively targeted PDT unconventional treatments do seem to possibly have a probability of enhancing treatment for metastatic melanoma within the near future of applied research.



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ORCID iD

Cherie Ann Kruger, PhD  <https://orcid.org/0000-0002-4556-9132>
Heidi Abrahamse, PhD  <https://orcid.org/0000-0001-5002-827X>

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